The acoustic space of pain: cries as indicators of distress recovering dynamics in preverbal infants

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Cries as indicators of distress recovering dynamics in preverbal infants

Alexis Koutseff[a], David Reby[b], Olivier Martin[c], Florence Levrero[a], Hugues Patural[d] and Nicolas Mathevon[a]*

[a]*Equipe Neuro-Ethologie Sensorielle, ENES/Neuro-PSI CNRS UMR9197, University of Lyon/Saint-Etienne, Saint-Etienne, France*

[b]*School of Psychology, University of Sussex, Brighton, UK*

[c]*Hôpital Privé de la Loire, Saint-Etienne, France*

[d]*SNA-EPIS EA4607, University of Lyon/Saint-Etienne, Saint-Etienne, France*

*Corresponding author. Email: mathevon@univ-st-etienne.fr.

14 **Abstract**

15 Crying is a vital built-in survival mechanism for the Human baby. Yet both the information
16 carried by cries and the factors driving the perception and reaction of adult listeners remain
17 under-investigated. Here, we contrasted the relevance of psychoacoustic vs. acoustic
18 evaluation for the assessment of distress levels in babies' cries recorded during baths and
19 during an immunization event. Parents asked to rate the level of distress experienced by
20 babies from listening to their cries attributed lower pain ratings to mild discomfort (bath) than
21 to distress (vaccination) cries but failed to discriminate between different putative levels of
22 pain experienced during different vaccination sequences. In contrast, vocal "roughness", a
23 composite acoustic factor characterising the level of aperiodicity of the cries, not only differed
24 between mild discomfort and distress cries but also between the levels of pain experienced
25 during the different vaccination sequences. These observations suggest that acoustic analyses
26 are more powerful than psychoacoustic evaluations for discriminating distress levels in
27 babies' cries, and opens the way for the design of a tool based on the acoustics of cries for
28 assessing and monitoring pain levels in preverbal infants.

29

30 **Keywords:** Infant cries, acoustic analysis, pain correlates, parental behavior, human baby

31

Introduction

Pain assessment is an essential aspect of paediatric care (AAP Committee 2016; Stevens et al. 2007). Yet while self-reporting is routinely used to evaluate pain in adults and older children (Breivik et al. 2008), it cannot be used with pre-verbal infants (Hummel and van Dijk 2006). The visual scoring of behavioral cues (facial and body movements) by trained caregivers can provide immediate information but its accuracy is highly limited by inter and intra-observer variability (Bieri et al. 1990; Cong et al. 2013; Hicks et al. 2001; Taddio et al. 2009). While the recent development of clinical devices measuring physiological markers (e.g. heart rate variability) represents a considerable improvement for the assessment of neonates' pain (Faye et al. 2010), these systems typically require monitoring devices (e.g. photopletysmographic sensor or ECG) connected to a human-computer interface, restricting their use to highly-equipped environments (Butruille et al. 2015).

Because crying in human infants is normally triggered by pain, discomfort, hunger or separation from parents or other caregivers (Lester and Boukydis 1985; Soltis 2004), its potential to carry information about the baby's distress makes it an obvious candidate for assessing pain levels in neonates (Barr et al. 1996; Barr et al. 2000; Bellieni et al. 2004; Boukydis and Lester 2012; Brown 1987; Gibbins and Stevens 2001; Gibbins et al. 2008; LaGasse et al. 2005; Ludington-Hoe et al. 2002; Weissman et al. 2009). Adult listeners discriminate between levels of crying intensity and rate longer cries with shorter silences and a higher fundamental frequency as more aversive (Cecchini et al. 2010; D'Odorico 1982; Green et al. 1987; Gustafsson et al. 2013; Lester et al. 1992; Zeskind et al. 1992; Zeskind and Lester 1978; Zeskind and Marshall 1988). However, the reliability of pain assessment using cries by listeners remains to be demonstrated, as most previous studies have investigated cries produced during single acute painful events (e.g. circumcision, heel lance; Porter et al. 1986; Craig et al. 1988) even though factors such as its duration, origin or location may modulate

responses to pain (Cong et al. 2013). Previous studies have investigated the modulation of specific acoustic features of infant cries (e.g. amplitude of formants, indexes of spectral slope, Fuller 1991; distribution of energy among the frequency spectrum, Fuller and Horii 1988; fundamental frequency, spectral form, absolute intensity, Bellieni et al. 2004). However, there is a lack of comprehensive investigation of the acoustic structure of cries across contexts differing in the intensity of the distress experienced by the baby.

In the present study, we recorded crying babies in different conditions assumed to elicit a range of discomfort and distress levels, and analyzed the acoustic structure of cries. We then investigated the relative effectiveness of perceptual (assessment by human listeners) versus acoustical (signal analysis) methods for evaluating the pain levels encoded in the recorded cries.

Methods

Study design

We recorded cries produced by babies during two main contexts: mild discomfort cries given during bathing at home (control condition), and pain cries during two distinct vaccination sequences in the pediatrician's medical office potentially associated with different levels of distress (Ipp et al. 2009). We then conducted a psycho-acoustic study to investigate how parents assessed pain levels from these cries, followed by an acoustical analysis to contrast the cries' acoustic structure across the aforementioned contexts.

Participants

The study comprises 33 families living around Saint Etienne, France and followed by Dr Olivier Martin (OM). Informed consent was obtained from all parents. Inclusion criteria were

good health condition and absence of serious medical history. Babies who did not cry during either the bath given at home or the vaccination sessions performed in the pediatrician's office were not included. As a result, we included 26 babies from 24 families (14 girls and 12 boys; two families had dizygotic twins). Included babies were all full term and 60 ± 3.2 days old at vaccination day. Boys were 57.43 ± 1.50 cm height and weighted 5.09 ± 0.59 kg, as girls were 55.92 ± 1.47 cm height and 4.65 ± 0.44 kg. All 24 mothers (32.08 ± 3.25 years old) and fathers (33.9 ± 5.5 years old) took part in the psychoacoustic test.

Recording of babies' cries

Pain cries were first recorded during scheduled routine vaccination performed by OM in similar conditions on the doctor's examination table and without maternal holding, oral glucose administration or pharmacologic analgesia. Mild discomfort cries were then recorded during bathing, undressing or dressing by the parents at the baby's home (number of days between the recording of pain and bath cries = 6.9 ± 3 days). These cries are labelled as "bath" cries elsewhere in the manuscript.

During the vaccination session, two vaccines were injected: 0.5 ml of hexavalent DTPa-HBV-IPV/Hib vaccine (Vaccine Infanrix Hexa® GlaxoSmithKline Biologicals SA, Rixensart, Belgium, Dhillon 2010), and 0.5 ml of 13-valent pneumococcal conjugate vaccine (PCV13, Prevenar 13®; Pfizer Ltd, Sandwich, Kent, United Kingdom, Sucher et al. 2011). Each injection was made in a different buttock (alternated between babies), and separated by about 30 seconds. Cries were recorded with a microphone (Sennheiser MD42), positioned at about 30 cm from the baby and connected to a digital portable audio-recorder (Zoom H4n, sample rate = 48 kHz, uncompressed .wav files). Pain cries were recorded during and after each injection, until the baby stopped crying, in the presence of parents. Using Praat acoustic

editing and analysis software (Boersma 2001), we isolated four cries per baby: two cries randomly selected from the bathing recording (*bath* cries), the first cry given in response to the injection (*reaction* cry), and a second cry given approximately 6 seconds later (*recovery* cry). Each cry was a single continuous vocal utterance produced on a single exhalation (duration = 2.47 ± 1.44 seconds). For each baby we thus analysed two discomfort cries (bathing) and four pain (injection) cries: two cries for vaccine 1 (*reaction 1* and *recovery 1*) and two cries for vaccine 2 (*reaction 2* and *recovery 2*). The order of the vaccines was alternated: half of the babies received Infanrix first and the other half received Prevenar 13® first. Previous investigations of pain elicited by these vaccines based on behavioural scores (Modified Behavioral Pain Scale (MBPS), Parent Visual Analog Scale and Crying (yes/no)) suggest that Prevnar may cause more pain than Infanrix (contrasting first injections) and that infants experience more pain overall when the more painful Prevnar vaccine is administered first (Ipp et al. 2009). Importantly however, there is no established "best practice" nor official guidance regarding the order of these injections, neither from pharmaceutical providers nor from French medical authorities (Patural, *pers. com.*).

Psychoacoustic tests

Each parent was played the 6 cries from their own baby in a randomized order. Each testing session contained these instructions: "In this experiment, you will listen to your baby's cries", and, for each presented cry, "Please evaluate the pain expressed by the cry you hear on a scale from 1 to 7: 1 = No pain, 4 = Medium Pain, 7 = Extremely strong pain". Both parents of each baby were tested separately, at home, two weeks after bath recordings, and listened to the same cries presented in different orders (headphones: Sennheiser HD 25–1). All psycho-acoustic tests were performed using the Multiple Forced Choice experiment interface in Praat.

Acoustic analyses of cries

We measured the following acoustic variables using a purpose-made script in Praat: *duration*, *%voiced* (the percentage of the signal that is characterized by a detectable pitch using the '*To pitch…' Praat command*), *meanF0*, *maxF0*, *minF0, rangeF0, startF0 and endF0* (respectively the mean, maximum and minimum, range, start, and end F0 calculated over the duration of the signal) and *F0CV* (coefficient of variation of F0 over the duration of the signal, i.e the standard deviation of the frequency contour divided by the mean of the frequency contour. *F0CV* is a standardised index of variability around the mean). Inflection points were counted (as each change in the sign of the derivative the fundamental frequency contour) after two smoothing procedure: one with a relatively broad bandwidth ('*Smooth...'* command in Praat, bandwidth = 25) to suppress short-term frequency fluctuations while preserving minor intonation events (such as bleat-like frequency modulation), and a second with a narrow bandwidth (bandwidth = 2) to characterize strong F0 modulation (major intonation events). Both bandwidth parameters were chosen empirically as they provided adequate smoothing to characterise the two levels of intonation variation.

The number of inflection points was divided by the total duration of the voiced segments in each recording, resulting in two distinct indexes of F0 variation (*inflex25* and *inflex2*). We also characterised the variability of the cries' intensity by calculating *intCV*, the coefficient of variation of the intensity contour estimated using the "To intensity y" command in Praat. We quantified the periodic quality of the cries by estimating their harmonicity (*harm*, degree of acoustic periodicity, measured as the ratio of harmonics to noise in the signal and expressed in dB), an index of jitter (*jitter*, small fluctuation in periodicity measured as the average of 'local', 'rap' and 'ppq5' measures in Praat) and an index of shimmer (*shimmer*, small variation in amplitude between consecutive periods, measured as the average of 'local',

154 'apq5' and 'apq11' parameters in Praat). A final procedure characterized the spectral envelope
155 of the cry by applying a cepstral smoothing procedure (bandwidth: 900 Hz) to each crying
156 sequence, followed by the extraction of the first four spectral prominences (*SP1, SP2, SP3,*
157 *SP4*) of the resulting smoothed spectrum. Finally, nonlinear phenomena were characterised by
158 conducting a visual inspection of narrowband spectrograms (Figure 1). We measured: the
159 percentage of time with biphonation (*BP*), the percentage of time with periodic, vibrato-like
160 frequency modulation (*FM*), the percentage of time with subharmonics (*SH*) and the
161 percentage of time with deterministic chaos (*DC*).

162 In order to reduce our set of acoustic variables to a smaller number of uncorrelated factors, we
163 performed a principal component analysis (PCA) on all 23 acoustic variables extracted from
164 the full dataset of cries (see Supplementary Table 1 for mean ± SD of these 23 variables
165 depending on the recording condition). The Principal Component Analysis produced 8
166 components with eigenvalues > 1. The first two components had eigenvalues > 3, and
167 respectively explained 23 and 13 % of the variance. The loadings of the different acoustic
168 variables on the first two components are reported in Table 1 (see Supplementary Table 2 for
169 factor loadings on the remaining components)**.** Variable loadings indicate that the main
170 principal component PC1 can be interpreted as an index of *cry roughness*: cries with higher
171 PC1 values are less voiced, have a more variable F0, are less harmonic, have higher level of
172 jitter and shimmer and more occurrence of deterministic chaos. In contrast, the second
173 component PC2 characterises the *cry pitch*: cries with higher PC2 score have higher
174 fundamental frequency parameters (*meanF0, maxF0, rangeF0, startF0* and *endF0*).

175

176 ***Statistical analyses***

177 *Analysis of psycho-acoustic data*

In order to compare bath and vaccination cries, we contrasted the rating attributed by listeners to a cry emitted during the bath with the rating attributed to the *reaction cry* given in response to the first injected vaccine (either the Prevenar 13®or the Infanrix Hexa®, depending on which was administered first). To do this we conducted a Linear Mixed Model with *pain rating* as the dependent variable, *condition* (bath or vaccination) and *listener sex* as fixed factors, and *listener identity* as a random factor.

In order to test the effect of vaccine order and vaccine type on attributions of pain levels, a linear mixed model was performed using *pain rating* as the dependent variable, *vaccination sequence* (Prevenar 13®first or Infanrix Hexa®first), *cry position* (reaction or recovery cry), *vaccine type* (Prevenar 13® or Infanrix Hexa®) and *listener sex* as fixed factors, and *listener identity* as a random factor.

Analysis of acoustic data

We contrasted the acoustic structure of cries emitted during the bath, with that of the *reaction* cries given in response to the first vaccine (either Prevenar 13® or Infanrix Hexa®, depending on which was administered first). To do this we conducted a Linear Mixed Model with *cry roughness (PC1)* or *cry pitch (PC2)* as the dependent variable, *condition* (bath or vaccination) as a fixed factor, and *baby identity* as a random factor.

In order to test the effect of vaccine order and vaccine type on the acoustic structure of cries, we performed two linear mixed models using either *cry roughness (PC1)* or *cry pitch (PC2)* as dependent variables, *vaccination sequence* (Prevenar 13® first or Infanrix Hexa® first), *cry position* (reaction or recovery cry) and *vaccine type* (Prevenar 13® or Infanrix Hexa®)) as fixed factors, and *baby identity* as a random factor.

All models included main effects and their interactions. Statistics were conducted using SPSS 21 (IBM 2013).

Results

Psycho-acoustic data

Comparison of pain ratings by listeners between bath and reaction cries

There was a significant effect of the recording *condition* - bath or reaction cry to vaccine - on parents' *pain rating* ($F_{(1, 53.4)} = 37.1$, $P < 0.001$): pain ratings were lower for bath cries (*mean* ± *SE* = 2.86 ± 0.24) than for the *reaction* cry elicited by the first injected vaccine (*mean* ± *SE* = 4.84 ± 0.24) (Figure 2). Neither the *listener's sex* ($F_{(1, 41.6)} = 2.41$, $P = 0.128$), nor the interaction between *listener's sex* and *condition* ($F_{(1, 53.4)} = 0.087$, $P = 0.769$), had a significant effect on *pain rating*.

Effect of vaccination sequence, vaccine type, and cry position on pain ratings by listeners

There was a significant effect of *cry position* –reaction or recovery- ($F_{(1, 147.9)} = 29.04$, $P < 0.001$), but not of the *vaccination sequence* – Prevenar 13® first or Infanrix Hexa® first)- ($F_{(1, 42.9)} = 0.733$ $P = 0.397$), of *vaccine type* – Prevenar 13® or Infanrix Hexa®- ($F_{(1, 149.6)} = 0.478$ $p = 0.490$) or of *listener's sex* ($F_{(, 42.9)} = 2.431$ $P = 0.126$) on *pain rating* (Figure 3). None of the interactions terms were significant (all $P > 0.1$): parents rated the reaction cries as expressing a significantly higher level of pain (*Mean* ± *SE* = 5.1 ± 0.2) than the recovery cries (*Mean* ± *SE* = 3.8 ± 0.2), independently from the vaccination sequence and vaccine type (Figure 3).

Acoustic data

Comparison of cry roughness (PC1) and cry pitch (PC2) between bath and vaccination cries

There was a significant effect of *condition* (bath or reaction cry to vaccine) on *cry roughness* ($F_{1,25} = 84.2$, $P < 0.001$): *cry roughness* was lower in bath cries (*mean* ± *SE* = -1.42 ± 0.36) than in cries given in reaction to the first jab (*mean* ± *SE* = 2.04 ± 0.36) (Figure 4). There was no effect of *condition* on *Cry pitch* ($F_{1,25} = 1.16$, $P = 0.291$).

Effect of vaccination type, vaccination sequence and cry position on cry roughness (PC1) and cry pitch (PC2)

There were significant effects of *vaccination sequence* (Prevenar 13® first or Infanrix Hexa® first) ($F_{1, 23.4} = 5.412$ $P = 0.029$) and *cry position* (reaction or recovery) ($F_{1, 70.5} = 28.751$, $P < 0.001$) on *cry roughness* (Figure 5). Babies produced cries overall characterised by a higher index of roughness when Prevenar 13® was administered first (*mean* ± *SE* = 1.51 ± 0.46) than when Infanrix Hexa® was administered first (*mean* ± *SE* = 0.00 ± 0.46). *Cry roughness* was also higher in *reaction* cries (*mean* ± *SE* = 1.608 ± 0.36) than in *recovery* cries (*mean* ± *SE* = -0.10 ± 0.36) (Figure 5). However there was no significant effect of the *vaccine type* – Prevenar 13® or Infanrix Hexa® - ($F_{1, 98} = 0.173$ $P = 0.678$). Moreover, none of the interaction terms were significant (all $P > 0.1$). Finally, none of these factors or their interactions had a significant effect on *cry pitch* (all $P > 0.1$).

Acoustic trajectories of cries induced by successive vaccines

As displayed in Figure 6, the cries induced by successive vaccination events follow a specific trajectory within the acoustic space defined by the two first principal components PC1 and PC2 (calculated from the set of 23 measured acoustic features). In this acoustic space, bath cries ("Bath" ellipse, in black on Figure 6) are to the left (lower roughness) and below (lower

pitch) all vaccination cries, indicating that babies express less vocal tension. The spectrograms on Figure 6 show a bath cry (bottom left) and a reaction cry induced by Prevenar 13® (top right). The more harmonic structure of the bath cry (indicating mild discomfort) contrasts with the more nonlinear, chaotic structure characterizing the distress cry at the other extremity of the acoustic space. Strikingly, when Prevenar 13® is injected first (inducing firstly the "Prev1.Reac" cries), the cries' trajectory (in red on Figure 6) remains on the right of the acoustic space, with all successive cries expressing high levels of distress, even for the recovery cries induced by Infanrix Hexa® ("Inf2.Reco"). This area of the acoustic space (on the right on Figure 6) is characterized by higher roughness (mainly expressed by PC1) and to a lesser extent by a higher pitch (main factor loading on PC2). Conversely, when Infanrix Hexa® is injected first ("Inf1.Reac"), the cries' trajectory (in purple on Figure 6) is more central in the acoustic space, indicating less vocal distress. Specifically, the recovery cries are closer to the bath cries, with those induced by Prevenar 13® ("Prev2.Reco") being the closest. Thus, when the Prevenar 13® injection is done after a first injection of Infanrix Hexa®, the induced cries reveal lower levels of pain than when it is injected first. These results highlight the importance of vaccination order on the pain experimented by the baby: when the more painful vaccine is injected first, the less painful vaccine elicits a high level of pain than when the sequence is reversed. The potentiation effect of the first painful event over the subsequent ones is clearly revealed by the distribution of the cries along the *roughness* dimension (PC1 axis).

Discussion

We found that parents attributed lower pain levels to bath than vaccine cries, and to recovery than reaction cries, consistent with the reasonable assumption that bath and recovery contexts are respectively characterised by lower arousal and lower distress levels than vaccine and

reaction contexts. This is also in accord with our observations that the acoustic structure of cries varies between these contexts, with vaccine cries containing higher levels of roughness than bath cries, and reaction cries containing higher levels of roughness than recovery cries. This suggests that parents are able to spontaneously use acoustic features of cries in order to discriminate broad differences in distress levels in the cries of their own babies. Interestingly, there were no sex differences in pain levels perceived by parents, confirming recent observations that mothers and fathers perform similarly on tasks involving the extraction of information from the cries of their own babies (Gustafsson et al. 2013).

Parents did not, however, distinguish between pain levels elicited by different vaccine types or different vaccination sequences. Yet, previous observations indicate that Prevenar 13® is more painful than Infanrix Hexa® and that the order of vaccination has an effect on the overall distress experienced by the baby (Ipp et al. 2009). Concretely, our acoustic analyses reveal differences between these experimental conditions, with higher levels of roughness in cries following the injection of the Prevenar 13® vaccine than in cries following the injection of the Infanrix Hexa® vaccine as well as when the Prevenar 13® vaccine was injected first.

A key result of the present study is that acoustic variables characterising irregularities in vocal fold vibration (roughness) were better at discriminating between contexts than variables characterising the rate of vocal fold (F0 or pitch). This is line with a previous work showing that F0 does not show significant correlation with DAN score (a widely used composite measure of neonatal pain) for scores <8, but increases suddenly when DAN score is above 8 ("alarm threshold", Bellieni et al. 2004). In the present study, pitch levels (characterised by PC2) failed to discriminate between putative levels of discomfort and distress, despite extensive evidence that pitch influences perceived discomfort and distress in human babies' cries (Dessureau et al. 1998; Reby et al. 2016) as well as nonhuman infant vocalizations (Kelly et al. *in press*; Lingle and Riede 2014; Maruscakova et al. 2015). While F0 is expected

to increase with higher subglottal pressure and vocal fold stiffness and tension experienced in higher arousal contexts (Zeskind and Lester 1978), nonlinear phenomena (namely deterministic chaos, biphonation and subharmonics) are also likely to be more prevalent with increased vocal tension, as cries change from phonated to dysphonated and to hyperphonated (Lester and Boukydis 1985). Interestingly, recent research has shown that cry pitch is highly variable between individual babies (and a correlate of perceived femininity and masculinity), questioning the reliability of pitch as an absolute marker of distress (Kelly et al. 2017; Reby et al. 2016). In contrast, the present results show that the level of roughness not only differs between mild discomfort and distress (bath versus vaccine), but also differs between a range of distress levels (*reaction* versus *recovery* cries as well as *vaccine order*). This is consistent with observations that nonlinear phenomena and low frequency modulation, which affect perceived vocal roughness, are increasingly identified as markers of strong vocal tension, which may be characteristic of negative valence in high arousal contexts (Arnal et al. 2015).

Moreover, the present study highlights that combinations of acoustic markers linked to cry roughness can perform better than the parents (familiar with their own babies' cries) at discriminating between contexts. While we cannot exclude that caregivers or practitioners could be trained to identify specific features of cries, our results suggest that a simple and objective tool based on a limited set of acoustic variables characterizing roughness could be embedded in PC, tablet or smart-phone applications and used to monitor pain in babies and assist therapeutic practices.

Registration information

The research reported in the present paper was reviewed and approved by the Ethical Committee of the University Hospital of Saint-Etienne (Comité d'Ethique du CHU de Saint-

322 Etienne and the Commission Recherche de Terre d'éthique; Institutional Review Board:
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329

330 **Disclosure statement**

331 No potential conflict of interest was reported by the authors.

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FIGURES

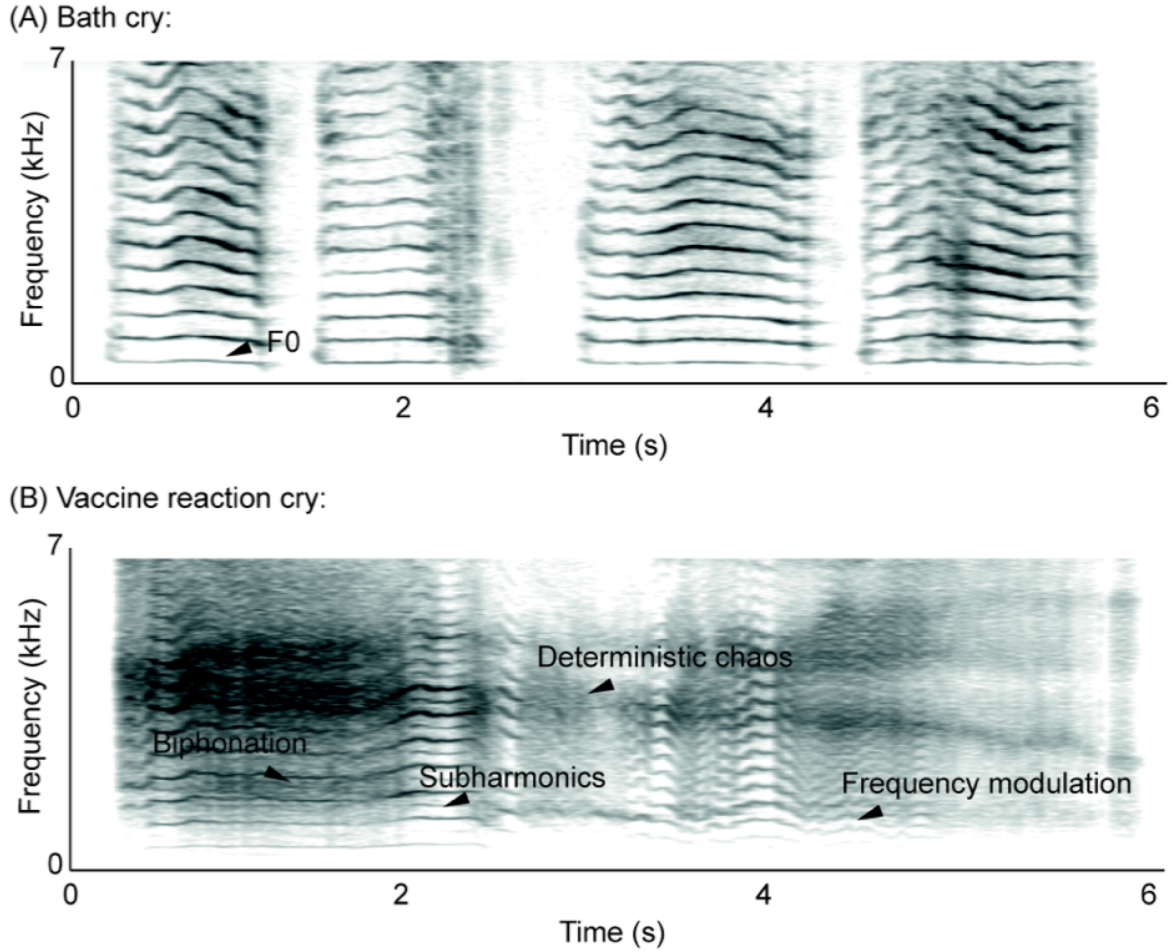

Figure 1. Spectrograms of a cry recorded during bath (A) and immediately after a first vaccine injection (B). While the bath cry shows a well-defined harmonic structure, the vaccine reaction cry is characterized by nonlinear phenomena responsible for vocal "roughness" (biphonation, subharmonics, deterministic chaos and vibrato-like frequency modulation).

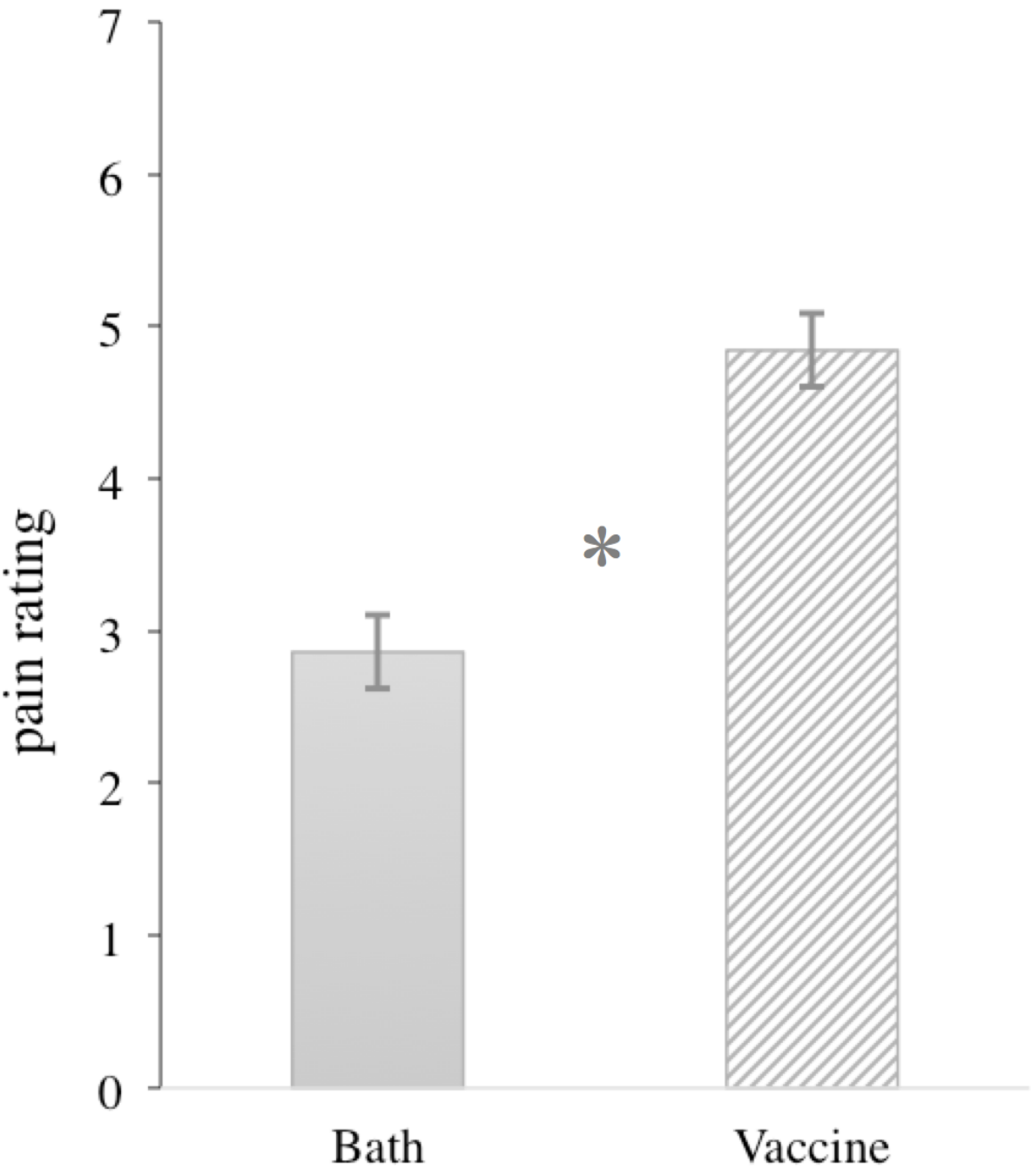

Figure 2. Effect of condition (bath vs. reaction cry to first vaccine) on parents' pain ratings (mean ± SE). Parents rate bath cries as expressing significantly less pain than vaccine reaction cries.

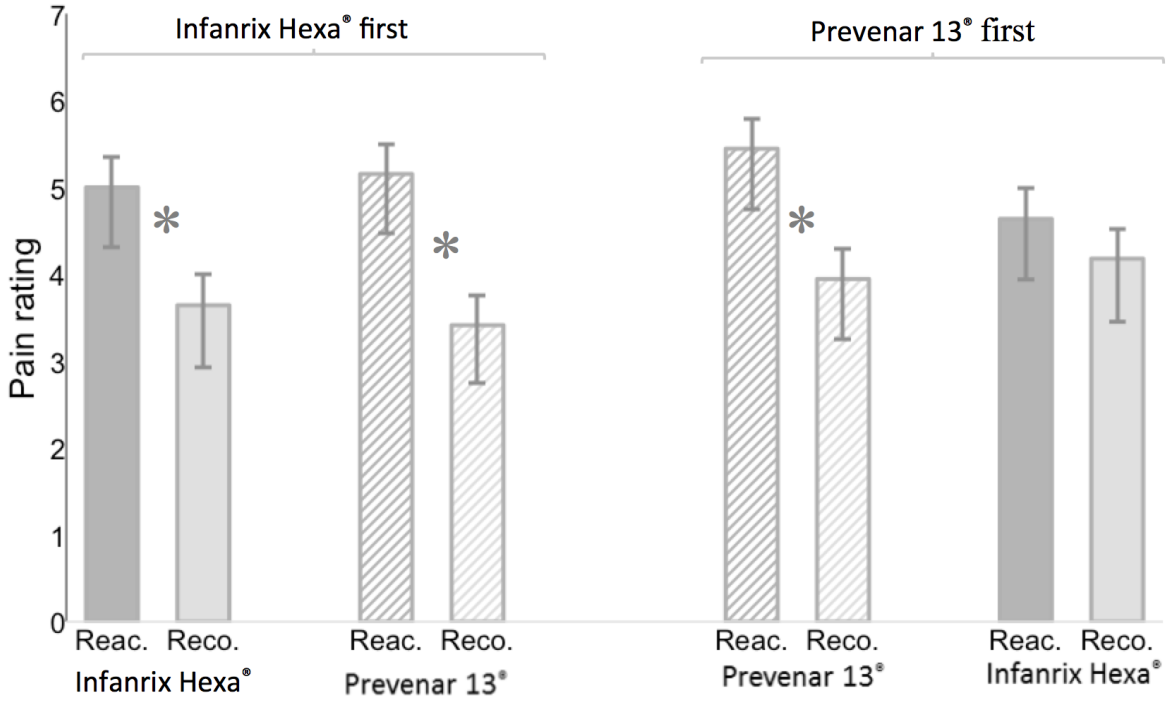

Figure 3. Effect of vaccination sequence, vaccination type and cry position on parents' pain ratings (means ± SE). Parents rate recovery cries as expressing significantly less discomfort than reaction cries, but do not attribute significantly different ratings between vaccination sequence or vaccine type.

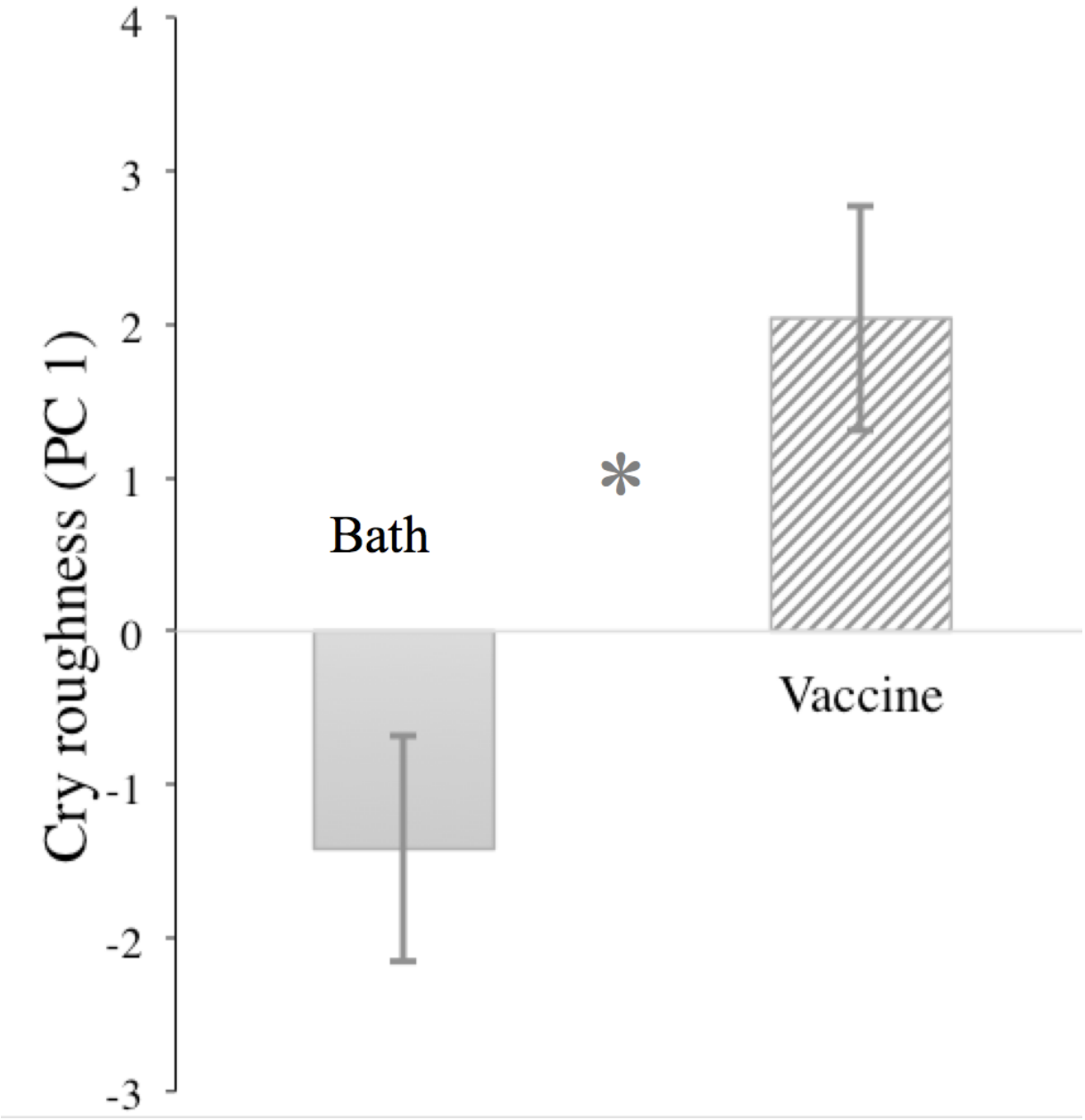

Figure 4. Effect of condition (bath vs. first vaccine reaction) on cry roughness (means ± SE). Vaccine cries have a significantly rougher quality than bath cries.

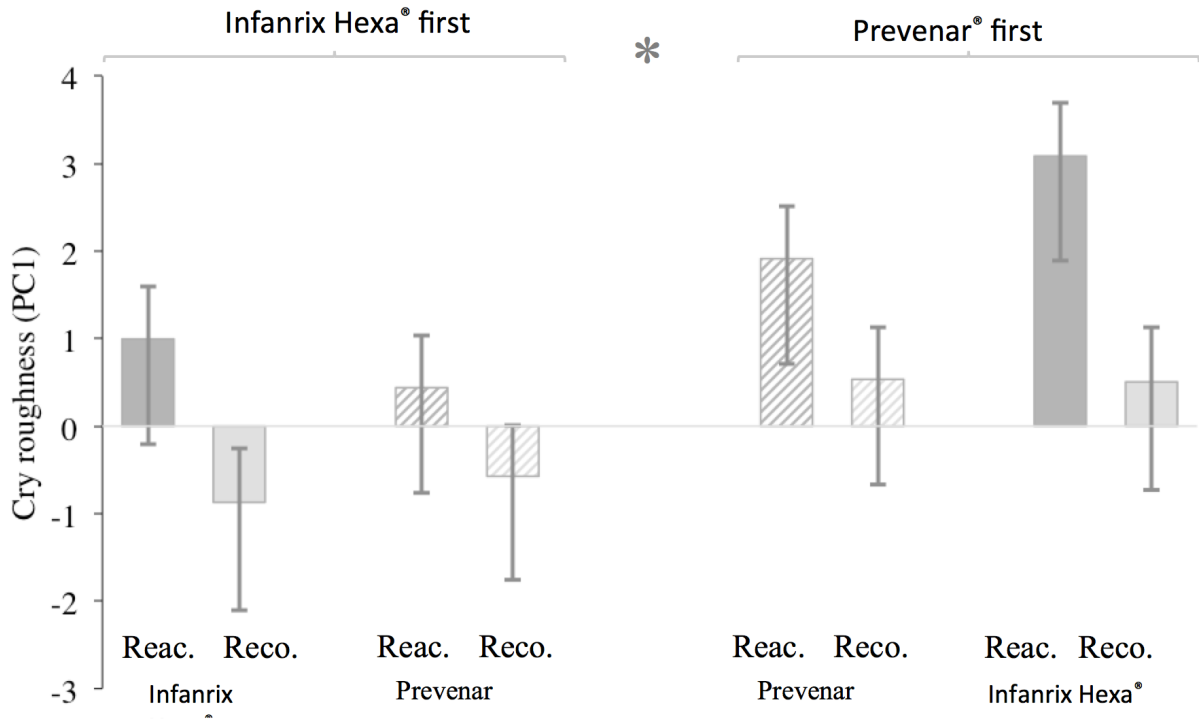

Figure 5. Effect of *vaccination sequence* (InfanrixHexa® first or Prevenar® first), *vaccination type* (InfanrixHexa® vs. Prevenar®) and *cry position* (reaction vs. recovery) on *cry roughness* (PC1: means ± SE). Cries are more aperiodic in vaccination sequences were Prevenar® is administered first. Reaction cries are also significantly more aperiodic than recovery cries.

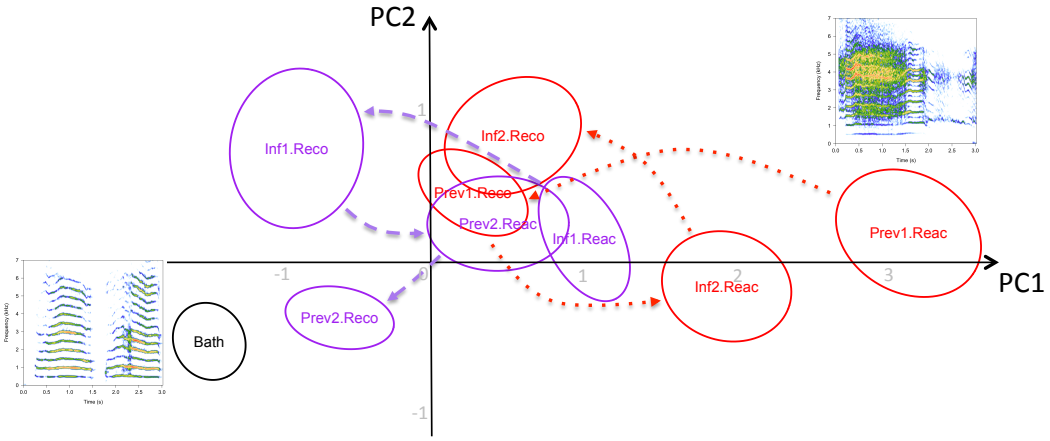

Figure 6. Trajectories of cries induced by successive vaccination events within a two-dimensional acoustic space. The principal components PC1 and PC2 have been calculated from a set of 23 acoustic features; PC1 represents *cry roughness* and PC2 represents *cry pitch* (see text for details). The ellipses are centered on the centroids and show one standard error of the mean of the distribution of recorded cries. In this acoustic space, bath cries ("Bath" ellipse, in black) are to the left (higher harmonicity) and below (lower pitch) of all vaccination cries indicating that babies express less vocal tension. When Prevnar is injected first (inducing firstly the "Prev1.Reac" cries), the cries trajectory (in red) stays on the right of the acoustic space, with all successive cries expressing high levels of distress, even for the last, recovery, cries induced by Infanrix ("Inf2.Reco"). Conversely, when Infanrix is injected first ("Inf1.Reac"), the cries trajectory (in purple) moves to the left of the acoustic space, indicating less distress. Specifically, the recovery cries are closer to the "bath" cries, with those induced by Prevnar ("Prev2.Reco") being the closest. The spectrograms show a bath cry (botton left) and a reaction cry induced by Prevnar (top right). The harmonic structure of the bath cry indicating mild discomfort contrasts with the biphonation, subharmonics, and deterministic chaos characterizing the distress cry at the other extremity of the acoustic space.

Table 1 - Factor loadings on the first two principal components calculated from the acoustic parameters describing babies' cries.

Acoustic parameters	PC1 % of variance = 23% Eigenvalue = 5.3	PC2 % of variance = 13% Eigenvalue = 3.0
%voiced	**-0.64**	-0.12
duration	0.45	-0.12
mean F0	-0.37	**0.78**
max F0	0.30	**0.87**
min F0	-0.51	**0.44**
rangeF0	0.53	**0.62**
startF0	0.01	**0.60**
endF0	-0.13	**0.71**
F0CV	**0.77**	0.12
inflex25	0.13	-0.12
inflex2	0.07	0.08
intCV	0.13	0.24
harm	**-0.81**	0.07
jitter	**0.80**	0.11
shimmer	**0.63**	0.05
SP1	0.34	-0.09
SP2	0.54	-0.07
SP3	0.53	-0.06
SP4	0.52	-0.15
SH	0.15	0.08
BP	0.16	0.16
FM	0.06	-0.20
DC	**0.75**	-0.04

Supplementary Table 1 - Mean± SD of the measured acoustic variables.

Acoustic parameters	Recording condition								
	Bath	**Prev1.Reac**	**Prev1.Reco**	**Inf2.Reac**	**Inf2.Reco**	**Inf1.Reac**	**Inf1.Reco**	**Prev2.Reac**	**Prev2.Reco**
%voiced	.85±.09	.54±.16	.83±.11	.69±.20	0.79±0.14	.68±.12	.84±.09	.76±.17	.86±.09
duration	1.77±0.63	4.23±1.97	2.19±0.56	3.20±1.39	1.91±1.22	3.94±1.43	1.94±.72	3.41±1.79	1.85±1.22
mean F0	436±57	417±108	456±80	406±109	488±91	452±110	474±78	442±71	416±58
max F0	563±84	666±114	674±73	643±120	682±162	648±139	658±199	635±95	595±82
min F0	269±62	232±59	242±53	241±59	248±37.7	255±82	287±79	262±71	253±33
rangeF0	293±99	435±88	432±66	402±102	433±168	392±91	371±213	373±122	342±103
startF0	386±67	455±131	483±66	404±103	422±26	414±93	476±190	467±113	437±80
endF0	384±106	330±107	355±120	359±116	436±130	354±72	421±148	413±105	386±98
F0CV	.15±.06	.27±.07	.23±.08	.23±.09	.23±.10	.21±.06	.16±.09	.20±.09	0.17±0.08
inflex25	12.2±3.7	11.5±4.6	15.5±4.4	14.4±4.08	13.6±5.5	13.1±4.7	13.1±6.3	15.2±4.9	14.9±3.8
inflex2	1.29±0.71	1.20±0.71	1.58±1.07	1.27±0.52	1.39±0.56	1.53±0.61	1.35±0.86	1.50±0.71	1.42±.59
intCV	1.32±.27	1.47±0.25	1.16±0.25	1.36±0.20	1.18±.0.29	1.50±0.27	1.32±0.30	1.39±0.25	1.18±0.34
harm	16.0±4.3	9.83±5.59	13.7±4.9	9.42±5.21	12.4±5.2	12.8±5.2	16.5±7.8	13.7±5.8	15.1±4.7
jitter	.008±.006	.021±.011	.013±.006	.019±0.01	.015±.008	.013±.001	.008±.005	.011±.01	.012±.008
shimmer	.057±.026	.093±.021	.06±.02	.09±.03	.07±.02	.07±.03	.05±.03	.069±.03	.073±.03
SP1	1450±577	1725±610	1526±396	1553±482	1590±641	1674±475	1335±409	1478±452	1235±405
SP2	3053±1022	4593±1947	3376±1408	4506±2707	3773±1892	3963±1480	3817±1747	3743±1590	3274±1638
SP3	5523±1486	7131±2512	5927±2275	7171±3000	6249±2859	6434±1848	6860±1958	6752±1666	6214±1990
SP4	8204±2661	10462±3219	8907±3145	10029±3606	8610±3397	9559±2895	9920±2441	10359±3227	9079±2535
SH	2.71±6.9	42.6±135	3.86±7.72	7.19±16.1	8.74±12.5	5.04±9.14	1.23±3.97	3.45±6.38	3.12±6.29
BP	0.92±3.42	1.69±3.53	1.85±5.20	4.71±10.2	0.80±2.87	4.19±9.16	.77±2.66	2.19±8.21	0.0±0.0
FM	5.02±14.3	9.17±12.8	7.04±12.8	3.84±8.2	9.37±20.6	13.4±20.3	4.24±7.99	16.7±22.1	7.4±20.0
DC	4.0±9.03	37.7±31.9	21.5±25.7	21.4±24.3	19.1±26.1	17.5±18.1	10.0±10..2	11.1±18.9	7.8±13.9

Supplementary Table 2 - Factor loadings on the principal components PC3 to PC8.

Acoustic parameters	PC3 % of variance = 11.2% Eigenvalue = 2.6	PC4 % of variance = 8.7% Eigenvalue = 2.0	PC5 % of variance = 6.4% Eigenvalue = 1.5	PC6 % of variance = 5.1% Eigenvalue = 1.2	PC7 % of variance = 5.0% Eigenvalue = 1.1	PC8 % of variance = 4.5% Eigenvalue = 1.0
%voiced	-0.02	0.60	-0.32	-0.03	0.12	0.06
duration	-0.11	0.11	0.60	0.22	0.10	-0.24
mean F0	0.24	-0.03	-0.02	0.06	0.06	-0.14
max F0	0.87	0.21	0.10	-0.06	0.10	-0.13
min F0	0.24	-0.25	-0.08	-0.19	-0.19	0.04
rangeF0	-0.19	0.32	0.13	-0.29	0.19	-0.14
startF0	0.11	0.14	0.21	0.09	-0.26	0.26
endF0	0.10	0.09	-0.19	0.09	-0.21	-0.07
F0CV	-0.24	0.18	0.10	-0.28	0.11	0.01
inflex25	-0.21	0.72	-0.11	0.28	-0.04	0.22
inflex2	0.01	0.36	-0.49	-0.17	-0.02	-0.12
intCV	0.14	-0.53	0.25	-0.01	0.26	0.21
harm	0.26	0.11	0.25	-0.23	0.16	0.08
jitter	-0.28	-0.14	-0.27	0.14	-0.03	0.12
shimmer	-0.29	-0.14	-0.26	0.41	-0.09	0.07
SP1	0.67	0.03	-0.03	0.16	-0.09	-0.26
SP2	0.71	0.14	-0.07	0.01	0.04	0.10
SP3	0.75	0.08	-0.08	-0.01	0.11	0.11
SP4	0.67	0.14	0.01	-0.04	0.08	0.08
SH	-0.02	0.07	0.23	-0.29	-0.49	0.63
BP	-0.17	-0.10	-0.16	0.23	0.67	0.41
FM	-0.08	0.51	0.51	0.43	-0.04	-0.04
DC	-0.14	-0.21	-0.12	-0.14	-0.31	-0.20